

Applicant: Garrity et al.
Serial No.: 09/761,969
Filed: January 16, 2001
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Amendments to the Specification

Please add the following new paragraphs after the paragraph beginning at page 6, line 21.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is an illustration of general assays (A through H) in which a releasing composition may be employed.

FIG. 1B is a legend of the elements of FIG. 1A.

Please replace the paragraph beginning at page 10, line 1 with the following amended paragraph:

In one embodiment, the releasing component further comprises a surfactant and the like. Without wishing to be limited by any theory or mechanism of operation, it is believed that the surfactant and the like of the present invention is at least effective to block the vitamin D component from being attached to lipids, proteins and the like. In a preferred embodiment, the surfactant is TWEEN-20 or TRITON X-100 ~~tween-20 or triton X-100~~. The releasing composition of the present invention may include about 0.01 to about 0.1% of a surfactant. For example, 0.05% of TWEEN-20 ~~of tween-20~~ may preferably be employed in the present invention. In another preferred embodiment, the releasing component is substantially free of a surfactant.

Please delete Table I beginning at page 19, line 1 and ending at page 20.

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Please replace the paragraph beginning at page 24, line 12 with the following amended paragraph:

Intra Coefficient of Variation (CV) studies for the determination of the 25-OH-D were conducted with various systems, including a system using a kit of the present invention in conjunction with Nichols automated assay machine, the Nichols Advantage. The studies using the kit of the present invention and Nichols Advantage automated technique involves obtaining a patient sample and performing the assay 20 times. The results are shown in Table I Table-II.

Please replace the paragraph beginning at page 25, line 1 with the following amended paragraph:

Table I Table-II

Please replace the paragraph beginning at page 26, line 17 with the following amended paragraph:

About 1 mg of Vitamin D binding protein, DBP, is buffer exchanged three times with 100 mM PBS at pH 8.2. The final volume was 200 uL. Succinyl-amino-buty-ethyl-isoluminol NHS ester (4.6 mg) is dissolved in DMF. The isoluminol (17 uL) is added to the DBP and the mixture is maintained at room temperature for about 1 hour with occasional shaking. The unreacted label is removed by size exclusion chromatography using SEPHADEX-G25 Sephadex-G25 (23 X 1 cm) and 100 mM PBS, pH 6.0 as the mobile phase.

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Please replace the paragraph beginning at page 27, line 10 with the following amended paragraph:

About 2.0 mg of DBP is buffer exchanged with 20 mM bicarbonate at pH 9.6 three times to give a final volume of 1.0 mL. Two aliquots of sulfo-NHS-LC-biotin (Pierce) are dissolved in water to give a 1 mg/mL solution just prior to addition. The first aliquot (10 uL) is added and the reaction is maintained at room temperature for 5 minutes. The second aliquot (10 uL) is added and the reaction proceeds for an additional 4 minutes. The unreacted biotin is removed by size exclusion chromatography using SEPHADEX G-25 ~~Sephadex G-25~~ and 100 mM PBS, pH 7.4 as the mobile phase.

Please replace the paragraph beginning at page 27, line 25 with the following amended paragraph:

2.5 mg of antibody is buffer exchanged into 20 mM bicarbonate buffer, pH 9.6, three times to give a final volume of 1.75 mL. Sulfonylchloride acridinium ester is dissolved in sufficient acetonitrile to give a 1.75 mg/mL solution. The acridinium ester (52.5 uL) is added to the antibody and the reaction mixture is maintained at room temperature about 0.5 hour. The unreacted label is removed by size exclusion chromatography using SEPHADEX G-75 ~~Sephadex G-75~~ and 100 mM PBS, pH 6.0 as the mobile phase.

Please replace the paragraph beginning at page 28, line 2 with the following amended paragraph:

Anti-DBP (Dako) is buffer exchanged to yield 2.2 mg of protein in 1.1 mL of pH 9.6, 20 mM bicarbonate buffer. A solution of sulfonylchloride acridinium ester (1.6 mg/mL) is in acetonitrile. The acridinium (4.4 uL) is added to the antibody and the reaction mixture is maintained at room temperature for 15 minutes. A second aliquot of acridinium (4.4) uL is added for and additional 15 minutes. The unreacted label is removed by chromatography on a mixed bed column (SEPHAROSE 6B/SEPHADEX G-75, 1.5 cm X 48 cm) (~~Sepharese 6B/Sephadex G-75, 1.5 cm X 48 cm~~).